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Research Article

Development and evaluation of letrozole loaded proniosomes as drug delivery system

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ABSTRACT

Maltodextrin was used as a carrier for the preparation of letrozole proniosomes. Slurry method was applied for the preparation with various ratios of cholesterol and surfactant. FTIR analysis, Scanning Electron Microscopy (SEM), angle of repose, encapsulation efficiency, and *in vitro* release of drug release were performed for prepared proniosomes. Stability study for the best proniosome formulation was carried out to determine the drug leaching during storage. Surface of carrier coated by surfactant has confirmed by SEM analysis. Entrapment Efficiency of LS207 was higher among the all 10 formulations. Formulation which contained molar ratio of 0.4:0.6 (span20:cholesterol) gave maximum encapsulation efficiency of 76.32 ±0.93% and the maximum drug release of 99.34% as compared to other compositions. Kinetic release study shown that optimized formulation LS207 exhibited zero order release& super case II transport diffusion based on the peppa's plot release exponent value. At refrigerator condition optimized proniosome formulation was appropriately stable over a period of three months.

Keywords: Malto dextrin; Span20; Slurry method; encapsulation efficiency.

INTRODUCTION

Researchers paid more attention to niosomes due to its targeting capacity. These had several advantages compared with conventional delivery systems (Schreier H et al., 1994; Baillie A et al., 1985). Studies revealed that niosomes have merits over liposomes even both systems could entrap hydrophilic and hydrophobic drugs (Yoshioka T et al.,1994;Uchegbu IF et al.,1995; Uchegbu IF et al.,1998). Due to its non-ionic nature niosomes shown less toxicity and it is not necessary to apply special efforts for the preparation (Vyas Jigar et al., 2011). However, while developing any formulation stability should be concerned primarily even though so many merits associated with niosomes like less expensive and chemical stability over liposomes (Namdeo A et al., 1999).

Proniosomes are carriers prepared by coating with surfactants in the form of free flowing dry powders. When rehydrated proniosome with hot water and apply agitation, these could form multi lamellar suspension which can suitably administer orally (Chengjiu Hu et al., 1999; Mokhtar M et al., 2008).

Physical stability problems associated with niosomes like leaking, aggregation and fusion were minimized by proniosomes (Blazek-Welsh AI et al., 2001; Vyas and khar., 2007) and other significance of proniosomes are perfect dosing, storage, easy transport. So that for administering different type of compounds proniosomes is considered as ideal system.

Dry proniosome is more stable than conventional niosomes. While seeing release of drug, proniosomes have more or less similar results. When comparing size of proniosome derived niosomes with normal niosomes proniosomes showed better results. So the release would be good (Almira I. Blazek Welsh et al., 2001; Rhodes D G et al., 1998; Vora B et al., 1998; Fang JY et al., 2001; Alsarra IA et al., 2005; Ankur Gupta et al., 2007).

Letrozole is a Non-Steroidal aromatase inhibitor. It is chemically known as 4, 4'- (1H-1, 2, 4-Triazol - 1ylmethylene) dibenzonitrile. It is the hormonal anticancer drug which can be employed to treat aromatase dependent carcinoma developed in breast. Quantity of estrogen secreted in the body could be reduced by administration of letrozole and also slow down or cease the breast tumour growth for which estrogen is necessary. The oral dosage form of letrozole offers uncontrolled delivery and release, lacks specificity, often leads to poor patient compliance, and results in major systemic side effects such as deep vein thrombosis, bone loss, and hypercholestremia, following long-term therapy (Cohen MH et al., 2002; Chen D et al., 2009). To increase target specificity, improve pharmacokinetic behaviour, it would be ideal if a sustained delivery system could be used for letrozole. Depend on this, proniosomes loaded with letrozole were prepared and evaluated. So in this research work tried

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to improve the therapeutic efficacy of formulated proniosomes.

MATERIALS AND METHODS

Letrozole received as a gift sample from Sun Pharmaceuticals Advanced Research Centre, Vadodara, India. Maltodextrin purchased from Himedia, Mumbai, India. Cholesterol and span20 were purchased from S.D.Fine Chemicals Limited, Mumbai, India. All other reagents used in this investigation were of analytical grade.

Formulation of Proniosomes

Based on the composition given in Table 1, using slurry method, proniosomes were formulated. Carrier maltodextrin should be taken in a round bottom flask. Then necessary amount of span20, cholesterol and drug should be added according to the formulae. These were dissolved by addition of chloroform. Further to make slurry, some more chloroform should be added in case of lower surfactant loading. Then in a rapid rotating flash evaporator, the round bottom flask was fitted and the solvent was evaporated at 60 rpm under reduced pressure at a temperature of 45±2°C , until the product become free flowing, dry condition. After that the obtained product were dried overnight at room temperature in a desiccator under vacuum. The obtained final preparation was termed as proniosomes which was stored in a sealed container at 5°C and kept it for evaluation process.

Evaluation of proniosomes

Angle of Repose Measurement

Angle of repose of dry proniosome powder was determined using funnel method. First pour the proniosome product into a funnel (13mm outlet orifice) which was fixed at a position 5cm above a level of plane surface. The poured powder flows down from the funnel to form a cone shape on the surface. The angle of repose was calculated by measuring the height of the cone and the diameter of the base with the help of calibrated scale.

Microscopy

After a suitable dilution, on a clean glass slide mounted the proniosome derived niosomes. Then for the determination of morphological character, it was observed under a microscope. proniosomes derived niosomes were mounted on glass slides and viewed under a microscope for morphological observation.

Size of Vesicle

With the help of same solvent system which was used for formulation, diluted the vesicle dispersions about 100 times. By using a particle size analyser vesicle size was measured.

Zeta Potential

One of the most important parameter is the particle charge which will assess the physical stability of any colloidal dispersion. The large number of particles was equally charged, then electrostatic repulsion between the particles was increased and thereby physical stability of the formulation was also increased. The purpose of zeta potential determination was to estimate the colloidal properties of prepared proniosomes. After diluting the proniosome derived nisomes, based on electrophoretic light scattering and laser Doppler velocimetry method zeta potential was determined using Malvern zeta sizer. By maintaining the temperature 25°c mean zeta potential values of preparations and charge on vesicles were obtained directly from the measurement.

Entrapment efficiency

By dialysis method, the unentrapped drug was first separated from proniosome derived niosome. The remained drug present in niosomes was estimated by complete vesicle disruption using Triton X-100.Then diluted with phosphate buffer pH 7.4 and filtered through whatmann filter paper. The percentage of drug encapsulation was calculated by the following equation:

$$\%EE = \left[\left(\frac{A_t - A_r}{A_t}\right)\right] \times 100\%$$

Where A_t is the concentration of total drug

A_r is the concentration of free drug

Number of vesicles per cubic mm

To assess the proniosome powder, number of vesicles formed after hydration was one of the main parameter. Phosphate buffer pH 7.4was used as a hydration medium. By utilizing haemocytometer and optical microscope the number of vesicles formed from proniosomes were counted in 80 small squares .The following formula was used for calculation

$$No.of\ niosomes\ per\ mm^3 = \frac{Total\ No.of\ niosomes\ counted \times dilution\ factor}{Total\ number\ of\ small\ squares\ counted} \times 4000$$

Scanning electron microscopy

It was one of the most important techniques used to analyz surface morphology. The morphology of surface like smoothness, roundness, aggregates formation and the size distribution of proniosomes were studied by Scanning Electron Microscope. Sprinkled the proniosomes onto the double sided tape which was affixed on aluminium stubs. Then placed it in the vacuum chamber of SEM.

Drug Release by *in vitro*

Open end cylinder method was used for the determination o*f in vitro* release of drug. Himedia dialysis membrane was used to cover one end of the cylinder. The prepared proniosome powder was placed over the membrane in the donar chamber. The donar chamber is then brought down to the vessels in the receptor

compartment which conain 100ml of phosphate buffer pH 7.4.So equal level of dissolution medi um and vesicle preparations where adjusted outside and inside respectively. At 37±0.5°C and stirring speed 50rpm, 2 millilitre of samples were withdrawn periodically up to 24hrs to analyse the drug release. Each time after withdrawal of sample, the medium is adjusted by replacing fresh dissolution medium to maintain the sink condition. The withdrawn samples were analysed spectrophotometrically. Every withdrawal was followed by replacement with fresh medium to maintain the sink condition. The samples were analysed spectrophotometrically.

Drug Release Mechanism by kinetic study

To understand the release mechanism of drug, the *in vitro* release of drug results were fitted with various kinetic equations such as zero order, first order, higuchi's model and korsmeyer peppa's model and the exponent n was calculated through the slope of the straight line.

$$\frac{X_t}{X_\infty} = ktn$$

Where X_t is amount of drug release at time t, X_∞ is the overall amount of the drug, k is constant, and *n* denote the release exponent value which shows the mechanism of drug release. If the release exponent value i.e. *n* = 0.5 or less, then the drug release mechanism is Fickian diffusion, and if *n* have value in between 05. to 1.0 then the drug release follows non-Fickian diffusion.

Proniosome stability

The optimized proniosome formulation was stored in airtight sealed glass vials at different temperatures i.e, 5±3˚C, 25±2 ˚C and 40±2˚C. To assess the stability of formulation vesicle size, drug remain and *invitro* release were taken as parameter. Because if drug leakage occurred in formulation during storage indicated the instability leads to reduction in the percentage drug retained. At the end of 90th day, the vesicle size, drug remain and *in vitro* drug release were determined for proniosomes. (WHO, 2006).

RESULTS AND DISCUSSIONS

Letrozole proniosomes were prepared by slurry method using different proportions of non-ionic surfactant span20 and cholesterol. FT-IR study results revealed that all characteristic peaks of letrozole were appeared in the proniosomal formulation spectra, which indicated there was no phenomenal change in the position of peaks after successful method of preparation. From that coming to a conclusion that there was no chemical interaction and no change in stability of drug during the method of preparation. While comparing angle of repose of maltodextrin powder with proniosome formulation, angle of repose of proniosome powder was nearly similar to that of maltodextrin powder. The appreciable flow property of prepared proniosome formulation was given in Table 2.

Niosomes were spherical and few being elongated shape while observed under microscope (Figure1). The smaller size may result from efficient hydration of a uniform and thin film of surfactant mixture at low surfactant loading. Surface character and shape of letrozole proniosome (LS207) was analysed by SEM has given in figure 2. Surfactant coating was confirmed from surface morphology and the average particle size was in the range of 650±0.42nm.

Zeta potential was determined as +32mv for formulation LS207. Zeta potential is a measure of net charge of niosome. When the charge was higher on the surface of vesicles leads to repulsive force. Due to repulsive forces between vesicles could avoid agglomeration and prevent faster settling. So evenly distributed suspension was obtained indicated that prepared proniosome formulation LS207 would yield better stable suspension.

The drug entrapped in proniosomes were found in the range of 42.26±0.77 to76.32±0.93% has given in Table 2.Higher entrapment efficiency was observed for higher concentration of surfactant which might be due to the high fluidity of the vesicles. It also affected by cholesterol content. For high cholesterol concentration, entrapment might be higher. Previous reports indicated that while increasing the cholesterol content entrapment efficiency also was increased.Span20 which has higher phase transition temperature, entrapment efficiency would be affected. Larger vesicle size was also a reason for higher entrapment of drug. Niosomes became less leaky while using cholesterol which abolished the gel to liquid phase transition of niosomes.

For all 10 prepared proniosome formulations, release study was carried out and given in figure 3. Linear release was found in most of the formulations and almost 90% release of drug was observed within 24hrs. Formulations (LS206, LS207) which had high cholesterol ratio shown sustain release. A cumulative release of 99.34% was given by the best formulation LS207 at the end of 18 hr.

For the determination of drug release mechanism, the *in vitro* release data was fitted in various kinetic models like zero order, first order, highuchi's plot and peppa's plot and given in Table 3.The formulations shown linearity and high correlation values, for zero order. From this it was assumed that from all the formulations drug release followed either near zero or zero order kinetics. Highuchi's plot correlation values lied between 0.9482 to 0.9775 which indicated the mechanism of release of drug from formulation was diffusion The release exponent value of korsmeyer-peppas plot revealed the fact that the drug release follows super case II transport diffusion.

Table 1: Formulation of Proniosomes

Sl.No	Formulation Code	Letrozole	Span20 in mg	Cholesterol in mg	Molar ratio	
					Span 20 in mM	Cholesterol in mM
1	LS201	2.5 mg	346.46	0	1.000	0.000
2	LS202	2.5 mg	311.814	38.665	0.900	0.100
3	LS203	2.5 mg	277.168	77.33	0.800	0.200
4	LS204	2.5 mg	242.522	115.995	0.700	0.300
5	LS205	2.5 mg	207.876	154.66	0.600	0.400
6	LS206	2.5 mg	173.23	193.325	0.500	0.500
7	LS207	2.5 mg	138.584	231.99	0.400	0.600
8	LS208	2.5 mg	103.938	270.655	0.300	0.700
9	LS209	2.5 mg	69.292	309.32	0.200	0.800
10	LS210	2.5 mg	34.646	347.985	0.100	0.900

Table 2: Characterization of Letrozole Proniosomes

Formulation	*Angle of Repose in Θ ± STD	Vesicle size in nm	Zeta Potenial in *mv*	*Entrapment Efficiency in % ± STD	Number of vesicle per mm^3X10^5
LS201	30°45'±0.52	110	+17	42.26 ± 0.77	3.6
LS202	31°27'±0.46	190	+21	48.04 ± 1.09	3.8
LS203	31°33'±2.26	240	+21	52.02 ± 0.36	3.6
LS204	31°50'±2.08	330	+24	56.34 ± 0.55	1.6
LS205	32°58'±0.64	470	+26	64.46 ± 0.90	1.2
LS206	31°17'±0.48	540	+30	71.54 ± 0.43	0.8
LS207	30°16'±0.73	630	+32	76.32± 0.93	3.9
LS208	30°58'±0.94	690	+21	70.44± 0.34	3.4
LS209	33°27'±0.41	670	+22	63.66± 0.08	2.9
LS210	33°25'±0.43	450	+16	61.06± 0.31	1.8

 # Maltodextrin angle of repose 30°06'±0.14 *n=3

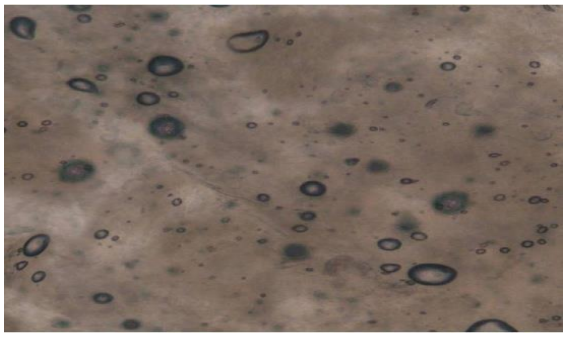

Figure 1: Photomicrograph of optimized proniosome derived niosome LS207

Figure 2: SEM image of proniosomal formulation LS207

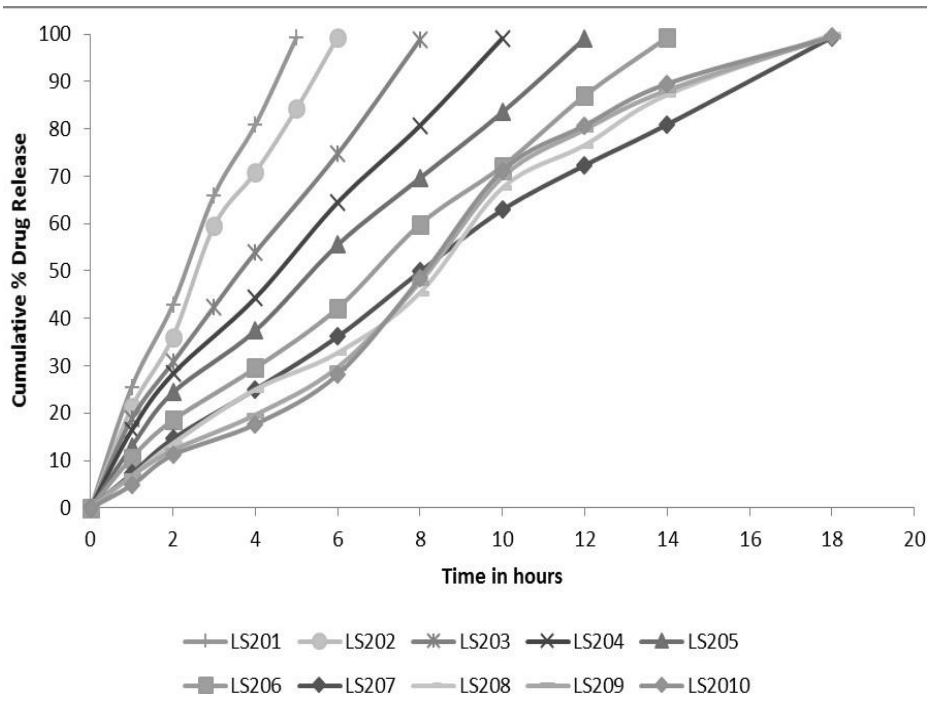

Figure 3: In vitro release of Letrozole proniosome formulations

Table 3: Kinetic data analysis of Letrozole proniosome formulations

Formula Code	Zero order		First Order		Higuchis model		Koresmayer & Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	*n*	R^2
LS201	18.5322	0.9982	0.8369	-0.8597	44.0017	0.9716	0.8512	0.9982
LS202	15.6182	0.9951	0.6896	-0.8510	41.1225	0.9715	0.8736	0.9967
LS203	11.3129	0.9997	0.4872	-0.8831	34.5283	0.9700	0.7956	0.9988
LS204	9.0747	0.9993	0.3869	-0.8699	31.4629	0.9775	0.7718	0.9987
LS205	7.6944	0.9989	0.3067	-0.8642	29.0634	0.9769	0.8026	0.9986
LS206	6.8913	0.9991	0.2669	-0.8497	27.2939	0.9661	0.8513	0.9970
LS207	5.5161	0.9972	0.2142	-0.8574	24.5723	0.9718	0.9109	0.9991
LS208	5.8394	0.9908	0.3090	-0.8115	25.6385	0.9616	0.9548	0.9966
LS209	6.0510	0.9843	0.2378	-0.8908	26.2650	0.9529	0.9942	0.9910
LS210	6.2262	0.9809	0.2406	-0.9009	26.8055	0.9482	1.1017	0.9916

Table 4: Stability study results for optimized letrozole proniosome

Temperature	Values obtained at zero time			Values obtaine at 90th day		
	Vesicle size in nm	Drug re-main in %	*In vitro* drug release in %	Vesicle size in nm	Drug re-main in %	*In vitro* drug release
5±3˚C	630	76.32	99.34	640	75.16	98.66
25±2˚C	630	76.32	99.34	650	73.02	96.41
40±2˚C	630	76.32	99.34	670	66.84	93.84

The remaining drug content and release study was estimated at the end of 90th day. It was monitored that the drug leakage from the vesicles was less at 5±3°C when compared with 25±2°C and 40±2°. This might be, upon storing leakage from vesicles occurred at high temperature due to phase transition of surfactant and lipid. So, confirmed that the stability of proniosomes could be maintained at 5±3°C followed by 25±2°C has given in Table 4.

CONCLUSION

Proniosome was a potential drug delivery system in which letrozole was successfully loaded using slurry method. Encapsulation efficiency and drug release rate was affected by the type of surfactant and cholesterol content. While comparing all molar ratio, LS207 which had a molar ratio of 0.4:0.6(span20:cholesterol) shown 76.32% encapsulation efficiency and 99.34% cumulative drug release after 18hr.Letrozole proniosomes were more srable over a period of 3 month while store at 5°C.From this work ,concluded that letrozole incor-

porated proniosome might be a controlled drug delivery system.

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